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Analyzing Adherence to Prenatal Supplements: Does Pill Count Measure Up?

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Analyzing adherence to prenatal supplements:

Does pill count measure up?

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A thesis submitted to the faculty of the Medical University of South Carolina in partial fulfillment of the requirement for the degree of Masters of Science in Clinical Research in the College of Graduate Studies.

Department of Biostatistics, Bioinformatics, and Epidemiology

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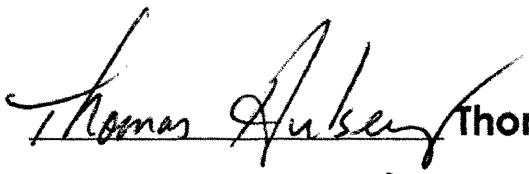
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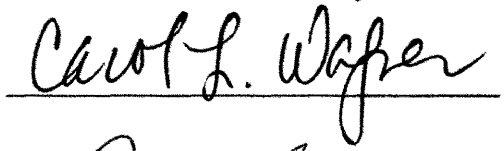
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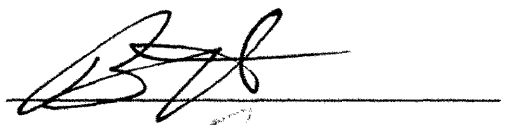
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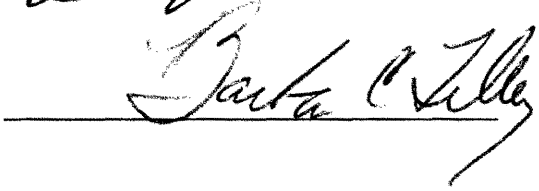
Barbara Tilley, PhD

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Abstract:

Background: Results of a trial of prenatal Vitamin D supplementation are analyzed for associations of pill count data with serum-based measures of adherence.

Objective: It is hypothesized that adherence as measured by pill count will have a significant association with serum-based measures of adherence.

Design: The study from which the data for this analysis were derived is a large randomized, controlled, and double-blinded trial of vitamin D supplements in pregnant women. The women have been stratified by race and randomized into three groups, which receive a 400IU (control), 2000IU, or 4000IU dose of vitamin D_3 once daily. Women enter the study at or before 12 weeks gestation and continue their supplementation throughout pregnancy and the first year postpartum.

Results: A series of 5 multivariate logistic regression models was created to examine whether mean percent adherence by pill count was significantly associated with the thresholds of adherence by serum 25(OH)D when controlling for race, dose, age, season at enrollment, and initial BMI. The analysis revealed that mean percentage of adherence by pill count was not a significant predictor of adherence by serum 25-OHD

at any time point. Odds ratios ranged from 0.9 to 1.1, and a significance level of 0.05 was not reached at any time point

Conclusions: In a multivariate logistic regression, there was not a significant association between adherence as defined by serum and mean percent pill count adherence for any time point.

Chapter I - Introduction:

Vitamin D is an important nutrient that is widely known to be vital to bone health and development, although it has recently been linked to other systems such as immune function(1). The two main human sources of vitamin D are sunlight exposure, which converts 7-dehydrocholesterol in the skin to vitamin D_3, and oral intake. Due to the limited dietary sources of vitamin D, serum vitamin D levels are primarily determined by sunlight exposure. The amount of vitamin D produced by a given amount of exposure is modified by skin pigmentation, with darkly-pigmented populations producing significantly less vitamin D than fair-skinned populations after exposure to similar conditions (2).

Existing guidelines recommend that pregnant women receive 200IU of vitamin D per day via an oral supplement (3). However, among African-American women who take daily supplementation in twice this amount, the rate of hypovitaminosis D is still 28% (4). The incidence of rickets, a pathology which can develop among children with hypovitaminosis D, is increasing, especially among darkly-pigmented populations (5-7). Studies are currently underway to determine the optimum supplementation dose of vitamin D among pregnant women (Wagner, Hollis, in progress). Once the optimum

dose is determined, the treatment effect of this intervention will be modified by the level of nonadherence among the patient population. The clinical implications of nonadherence are numerous and well-recognized (8).

Adherence to study medication is most often measured by a calculation of pill count. This is an inexpensive measure of compliance, but the data is unreliable and often missing. For example, in a 2005 study of prenatal supplements 56% of subjects remembered to return only one monthly pill bottle during the duration of the 2-3 month study (9). With this method, researchers are not only depending on patients to return bottles, but not to alter the number of remaining doses as well. In a 2001 study of protease inhibitor regimen adherence among HIV patients, pill count measures estimated adherence at 83%, but electronic monitors on the pill bottles revealed that true adherence was only 63% (10).

Lack of adherence dilutes treatment effects, which impacts the health of the target population. The purpose of this study was to define parameters for measuring adherence to vitamin D supplementation by serum metabolite levels in order to examine the association between adherence as determined by serum metabolites and adherence as determined by patients' pill counts.

Chapter 2 - Literature Review

Patient Adherence and Prenatal Vitamin D supplementation

Thesis Document: Analyzing adherence to prenatal supplements:

Does pill count measure up?

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Introduction:

This literature review encompasses two principal domains, patient adherence to prescribed medication regimens and the specific need for adherence to prenatal vitamin D supplementation. The thesis document which it supports is a study of measures of patient adherence in the context of a research project to determine appropriate

prenatal vitamin D supplementation dosage. Therefore, it is important not only to review known information on patient adherence in general, but also its specific importance in the clinical context of prenatal care.

Background:

Measures of patient adherence

One of the problems in researching any treatment is that of patient adherence to a prescribed regimen. How can one accurately measure the effect of an intervention if one is not certain that the patient is completing the treatment in the manner prescribed? In effect, this will dilute the true treatment effect and could also underestimate potential side effects of the dose studied. Also, it is difficult to take a lack of adherence into consideration in data analysis if one does not have a reliable measure of the prevalence and degree of these deviations from the intended intervention.

Lack of adherence is an important problem in the clinical realm as well (8). If an intervention is not having the desired effect, it is important for the clinician to know whether to attribute this lack of effectiveness to a lack of efficacy of the agent in a particular patient or simply patient non-adherence. If the clinician cannot adequately assess adherence, he or she may switch the patient to a new intervention with, for

example, more prominent side effects, when all that was needed was to reinforce the importance of taking the medication exactly as prescribed.

A previous review of literature on patient adherence found estimates of regimen adherence ranging from 19% to 100% (11). This could be due in part to the variety of methods and standards used. When considering adherence, it is important to keep in mind that it is not a homogeneous phenomenon. There are those subjects who never start the prescribed regimen, those who discontinue treatment prematurely, and those who continue treatment throughout the required time period but deviate from the prescribed regimen. This last group can be further subdivided into three overlapping categories. These include subjects that skip doses, subjects that do not take the regimen at the intended time intervals, and even those who take too many doses. The diversity of the phenomenon known as non-adherence makes the task of measuring it more difficult, as a single method could address some aspects of the issue but not others.

Many tools have been used to attempt to measure the lack of adherence in a patient population, each of which exhibits both strengths and weaknesses. We will undertake a review of recent and relevant literature in this arena.

In general, existing methods of determining adherence fall into the categories of indirect and direct measures. We will begin our review with indirect measures, including self-report, pill count, and pharmacy data. More direct and objective

measures reviewed will include medication event monitoring systems (MEMS), serum assays, and direct patient observation.

Indirect Assessment of Adherence:

Self-report

Self report is really a category for diverse forms of measurement, all of which are generally quite inexpensive and easy to use. This can be done via patient diary, interview, or a specifically-designed questionnaire. Among techniques, one specific interview question set yielded accuracy of about 75% (12); however, in this study the gold standard used for comparison was pill count, the flaws of which are discussed below. In addition, a 1998 article found that in an elderly population on long-term medications for chronic conditions, self-report and pharmacy claim data led to a higher estimation of adherence when compared to pill count (13).

The self-administered Basic Medication Questionnaire is another option; it includes not only questions about adherence, but also sections that focus on patient beliefs about the usefulness of the medication, potential adverse effects, and whether the subject is having trouble recollecting facts related to his or her level of adherence. The different domains of the BMQ showed an accuracy of 40-95% when results were compared to medication event monitoring system (MEMS) data, which was used as the

gold standard in this study (14). MEMS is a modern, electronic adherence assessment system which will be discussed separately later in this review.

A 2004 meta-analysis further analyzed self-report. In this case, all self-report data was compared to electronic monitoring as the gold standard. The concordance between the two types of measures was high in only 17% of cases (15). This meta-analysis also revealed another important finding. Interviews were much less likely to be concordant with electronic monitoring data when compared to other self-report formats, such as questionnaires and subject diaries. No studies included in the analysis showed a high concordance between interview results and electronic monitoring data, while data from questionnaires and subject diaries were highly concordant with the MEMS data in almost a third of the 16 such studies analyzed (15).

The advantages of patient diary and interview techniques are that they allow for assessment of a variety of forms of adherence. They not only measure whether the subject started and maintained the treatment, but can also assess missed doses, extra doses, and whether the subject kept to the intended schedule outlined in the prescribed regimen. Disadvantages of self-report techniques are lack of accurate records, inability to remember specific adherence behaviors, and possible fabrication of responses.

Pill Count

Pill count is an inexpensive but potentially unreliable way of measuring adherence. Due to its low cost and ease of calculation, this measure has been frequently used in the literature. There are many potential pitfalls to this method, including the important problem of missing data. For example, in a 2005 study of prenatal supplements 56% of subjects only remembered to return one of their monthly pill bottles during the 2-3 month duration of the study (9). With this method, researchers are not only depending on patients to return bottles, but to refrain from altering the number of remaining doses. This can be problematic, and often leads to overestimation of adherence. For example, in a 2001 study of protease inhibitor compliance among HIV patients, pill count measures put adherence at 83% while MEMS data showed a 63% adherence rate (10).

Another problem with measuring adherence by pill count is that cut-offs for adherence are relatively arbitrary. Frequently, adherence in a single patient is measured by the percent of expected doses that were taken over a given time interval. This is calculated by the following formula:

(# of pills dispensed - # of pills returned) / (# of elapsed dosing periods between dispense date and return date)

Many studies then convert this figure into a dichotomous variable, classifying patients as adherent or non-adherent based upon whether the calculated percentage falls above or below a pre-established standard. This cut-off for pill count adherence varies between studies. It is understandable that some conditions may require greater medication adherence than others, due to short half-lives of medications or the severity of complications that can develop. However, when the standard is set the statistical consequences must be considered. A higher standard would grant more sensitivity but less specificity, while a lower threshold would confer more less sensitivity but more specificity (11).

Measuring adherence by pill count has the previously mentioned advantages of low cost and ease of calculation, but it has disadvantages as well. Besides missing data, arbitrary cut-offs, and possible alterations by subjects, pill count data also do not detect all types of non-adherence. Pill count data would detect those patients who never began treatment, those who discontinued treatment, and those who skipped doses, but it offers no information on other aspects of non-adherence. For instance, pill count data cannot be used to assess whether a subject took the medication on the intended schedule. Also, many studies that measure adherence by pill count have no provisions for patients who took too many doses; this is also an important aspect of non-adherence, since overdose and dependence are important risks to many medications.

Pharmacy Data

Researchers seeking an alternative measure of adherence have analyzed pharmacy records to infer patient behavior based on the intervals at which refills were obtained. As with many of the other methods discussed in this review, the measures of refill compliance varied between studies. Refill compliance could be described as a continuous or dichotomous variable, much like pill count. It could also be measured over a single time point or over multiple time intervals. Finally, some analyses of refill compliance examine the time period when the medication was available, while others focus on the gaps in therapy inferred from pharmacy data (16).

The accuracy of pharmacy refill data is generally well-correlated with more direct measures of adherence. A review of pharmacy refill adherence studies showed that 3 out of 3 studies analyzed showed a significant association between refill data and subject serum drug levels, while 4 out of 5 studies showed a statistically significant association between refills and the anticipated effect of the medication (16).

As with other methods, pharmacy data has both advantages and disadvantages in measuring adherence. Strengths of this method include its relative lack of invasiveness; patients need only give their permission for these records to be accessed, and are not required to fill out forms, sit for interviews, or undergo venopuncture. It also has low cost and is easier than other methods to apply to a large population, which may be helpful in fields such as health services research. However it has disadvantages as well. Patients may refill their prescription at the assigned time even though they

have not taken all of the required doses. This is even more likely in recent years, as automatic refills and pharmacies-by-mail have become more common. Also, refill data do not offer any information on whether doses were taken according to the prescribed schedule. A final weakness of this method is that many patients refill before the assigned time interval has expired. Steiner and Prochazka found that different studies placed the prevalence of medication stockpiling between 4.8% and 35.1%. In this case, it is impossible to determine if the patient is consuming more than the prescribed dose, sharing doses with others, or simply hoarding the medication unless researchers follow-up with another inquiry method, such as interview.

Direct Assessment of Adherence:

Electronic Monitoring

Medication Event Monitoring Systems (MEMS) are a type of electronic monitoring of medication usage that functions by recording electronically the time and date that a pill bottle is opened, an inhaler is used, or a dropper dispensed. They represent a large expense for a study, but also provide specific information on the exact pattern of medication usage. The data obtained can reveal large discrepancies from that provided by self-report. For example, one 2007 study of schizophrenic patients (10) showed that the rate of subject non-adherence (defined as a dichotomous variable with an 80% adherence threshold) according to self report was only 3%, while MEMS data

revealed that the actual rate was 52%. This is an extreme example, likely affected by both the small number of patients (n= 52) and the strong association of their pathology with paranoia and disorganized cognition. However, effects in other populations are also significant. Another study demonstrated that comparison to electronic monitoring data demonstrated that 30% of entries in patient-kept regimen adherence diaries were in error (17).

One of the more remarkable results from studies in which electronic monitoring devices were used was the confirmation of the phenomenon of "white coat compliance," the desire to appear adherent at study visits. Subjects in one study were told that their inhalers were going to be weighed at visits to assess adherence, but were unaware that the dosing meter contained a chronolog that recorded the time and data of device actuations. 14% of subjects actuated the inhaler over 100 times in the three hours previous to a study visit, detecting deliberate medication dumping (18).

As an adherence assessment method, MEMS data are useful because, unlike many other measures, they allow the researcher to access information on whether the medication was taken on the prescribed schedule. It has been used as the gold standard of patient adherence measures in recent years due to the wealth of data provided by the monitoring devices. One of the weaknesses of this method is that it does not account for specific circumstances, such as two people in a household taking the same medication or a subject who dispenses all doses into a pill-case at the beginning of the week. Another disadvantage is that, while MEMS devices do offer objective data, they

do not prove that a dose was actually consumed each time that the bottle was opened, the inhaler actuated, and so on. Finally, electronic monitoring devices are costly. While they are feasible in the context of well-funded studies, MEMS devices are impractical for inclusion in many research budgets. The cost is prohibitive, which means that the use of electronic monitoring is limited to research, and not practical for the clinical context in most cases.

Serum Assays

Direct measures such as blood and serum assays are another objective way to measure patient adherence. Three types of serum measures are commonly used for this purpose: biologic markers with known half-lives added to medications, metabolites of medications and supplements, and levels of the medication in the serum. When using this method of assessment, it is important to consider that some compounds could also be obtained from other sources, such as dietary intake affecting caffeine levels, or, in the case of vitamin D supplementation, even exposure to sunlight. In the analysis, these other exposures that could affect serum levels of the compound of interest must be taken into account. Options to accomplish this include stratification by such possible confounders or adjustment for known exposures in the analysis.

Another key consideration in the use of serum levels as indicators of patient adherence is the half-life of the compound in question. Those compounds with a short

half-life lend themselves to an analysis of short-term adherence. While this is one indicator, one must keep in mind the phenomenon of "white coat compliance" discussed earlier. Compounds with longer half-lives can be used to assess long- or intermediate-term adherence. This gives a clearer picture of consistency of adherence than a short half-life compound. In considering the above concept, one can imagine a clinician who would like to track the adherence of his or her patient to their diabetes treatment. While the blood glucose reading taken that day gives a measure of current glucose control, a laboratory measurement of serum HbA1C measures the consistency of blood glucose control over a two or three month period (19).

Strengths of serum assays to determine adherence include their objectivity and usual predictability. The main difficulty is determining the appropriate cut-off point to use as the standard of adherence in a given population. Confounding factors such as age, gender, and BMI can influence serum concentrations of a metabolite (20-22). In addition, genetic polymorphisms could affect the rate at which metabolites are produced or cleared from the circulation. An extreme example is a population with ALDH2*2 alleles. In such a population, which includes about half of Chinese, Japanese, and Korean persons, one would make erroneous assumptions if attempting to estimate the amount of alcohol consumed by the level of acetaldehyde in each subject's serum (23). This is because this specific genetic polymorphism results in a deficiency in aldehyde dehydrogenase, the enzyme which converts the toxic ethanol metabolite acetaldehyde to acetic acid.

Direct Observation

Due to the weaknesses of the methods described above, direct observation is the only true way to assess all of the different possible permutations of patient non-adherence. Of course, this assessment method is very costly and labor-intensive, which usually limits its use to the inpatient setting. However, this method has also been used in other contexts. In a 2005 analysis of the effectiveness of Echinacea to prevent and ameliorate cold symptoms, subjects were sequestered in individual hotel rooms during a five day period (24). In this study, a method of liquid measurement which can be approximated to pill count had been used to measure adherence during an outpatient period. The authors noted that although the adherence was evaluated to be very good during the outpatient period, they were only certain of the adherence in the isolation phase, when direct observation was used at the time of each dose.

Importance of Vitamin D Supplementation

Vitamin D is an important nutrient that is widely known to be vital to bone health and development, although it has recently been linked to other systems such as immune function (1). Recently there has been a wealth of research into the beneficial effects of vitamin D. As one example, a new study shows that even after adjusting for other known risk factors, men who are deficient in 25-OH D are significantly more likely to suffer a myocardial infarction when compared to men with a 25-OH D serum

measurement that classifies as sufficient (25). Another new study, which examined data from 51 regions around the world, found a significant association between a low level of sun exposure and a high incidence rate of type 1 diabetes in children after controlling for per capita health expenditure in each country studied (26).

Vitamin D status is particularly important during fetal development and infancy. Previously established roles of adequate vitamin D in infants and children, particularly, were the integrity of bone development and prevention of rickets, a softening of the bones caused by inadequate vitamin D stores early in life, including during fetal development (7). It is important that pregnant women have adequate levels of vitamin D, since a strong relationship between maternal and fetal vitamin D status has been demonstrated in studies of human subjects (27).

The lack of adequate vitamin D status is currently a public health problem. Rickets and vitamin D deficiency are experiencing a resurgence (5, 6), most notably in the northern United States (28). This could contribute to racial health disparities, since over 40% of African-Americans of reproductive age suffer from hypovitaminosis D (4).

The two main human sources of vitamin D are sunlight exposure, which converts 7-dehydrocholesterol in the skin to vitamin D_3, and oral intake. Due to the limited dietary sources of vitamin D, serum vitamin D levels are primarily determined by sunlight exposure. The amount of vitamin D produced by a given amount of exposure is modified by skin pigmentation, with darkly-pigmented populations producing

significantly less vitamin D than fair-skinned populations after exposure to similar conditions (2).

Existing guidelines recommend that pregnant women receive 200IU of vitamin D per day via an oral supplement (3). However, among African-American women who take daily supplementation in twice this amount, the rate of hypovitaminosis D is still 28% (4). The incidence of rickets, a pathology which can develop among children with hypovitaminosis D, is increasing, especially among darkly-pigmented populations (5-7). Studies are currently underway to determine the optimum supplementation dose of vitamin D among pregnant women (Wagner, Hollis, in progress). Once the optimum dose is determined, the treatment effect of this intervention will be modified by the level of nonadherence among the patient population. The clinical implications of nonadherence are numerous and well-recognized (8).

Serum Assays to Assess Vitamin D Status

In assessing adherence, it is important to consider the characteristics of the population under study. The analysis in the attached paper focuses on pregnant women. Pregnant females are generally an adherent population, as was demonstrated by the success of a recent maternal folate supplementation program(29).

In order to assess the consistency of adherence to vitamin D supplements over a long period of time, the measurable metabolite with the longest half-life should be

measured. 25-hydroxyvitamin D (25[OH] D) has a 2-3 week half-life, making it ideal for this purpose.

Besides half-life, other important considerations raised previously with regards to assessing patient adherence via serum metabolite level include:

a. other possible sources of the compound in question,
b. possible factors that could affect the way the body stores and metabolizes the compound, and
c. how to establish an adequate cut-off value that indicates adherence.

Due to the low levels of vitamin D found even in supplemented food sources, 25(OH)D levels are principally determined by supplementation and the exposure to Ultraviolet B (UV B) radiation. The time of year that each subject enrolled is therefore important to consider in analysis of data, since daily sun exposure and clothing vary significantly by season. It is also important to remember that the amount of 25(OH)D that a person produces when presented with a certain amount of UV B exposure is significantly modified by skin pigmentation (2). Therefore, initial skin pigmentation is also important to consider when analyzing levels of 25(OH)D after supplementation.

Finally, BMI and body fat percentage have been found to have an inverse relationship with serum levels of vitamin D metabolites in several recent studies (21, 22, 30). Therefore, initial BMI was also important to consider in the analysis of adherence by serum metabolite levels.

The most difficult step in a serum assay analysis is the decision to establish a threshold serum value, above which subjects would be considered adherent. In this case, the foundation of the serum adherence thresholds of 25-OHD was grounded in the Heaney regression model (31). Of note, the predicted serum changes in 25(OH)D levels in the Heaney model were calculated on the basis of dose taken rather than dose prescribed. Therefore, they did make an effort to account for nonadherence in the study. It is also important to note the lack of available data concerning the effects of a given oral supplementation dose of vitamin D_3 on the serum 25(OH)D levels in females as opposed to males. The attached analysis used data from a cross-sectional study of a population (32) to adjust the predicted serum adherence thresholds rather than a controlled trial of supplementation doses. However, we feel that the present study was based on the best information currently available.

Conclusion

In summary, the measurement of patient adherence is a difficult process due to the unique advantages and disadvantages of each method by which it is assessed. However, it is worth the effort to use the most accurate method that is feasible given a current situation, given the important effects of patient adherence on both research studies and clinical practice.

Chapter 3 - Methods:

Study Design

The study from which the data for this analysis were derived was a large randomized, controlled, and double-blinded trial of vitamin D supplements in pregnant women. The women had been stratified by race and randomized into three groups, each of which received a 400IU, 2000IU, or 4000IU dose of vitamin D_3 once daily. Women entered the study at or before 12 weeks gestation and continued their supplementation throughout pregnancy and the first year postpartum.

Eligible subjects self-identified as Caucasian, African-American, or Hispanic/Latina and carried singleton pregnancies. All subjects were patients at obstetric clinics at the Medical University of South Carolina (MUSC, located in Charleston, SC, USA), and participated in a monthly study visit as an extension of their regular prenatal checkup. They were also asked to return to the clinic three times for study visits after the birth of their infants. At each study visit, levels of 25(OH) D and vitamin D3 were measured in patient serum. In addition, subjects were asked to bring their supplement bottle with them to their monthly visits, containing all unused supplements.

Note: The above-referenced research protocol was approved by the Medical University of South Carolina Institutional Review Board for Human Research.

Measures

Outcomes: Outcomes in this study were measured as percent adherence by pill count and adherence by serum metabolite levels. These values were determined at multiple time points for each patient.

Percent adherence by pill count

This was measured at each time point for each subject provided that pill count data were available. Subjects were asked to bring their supplement containers, containing all unused doses, with them to each monthly study visit. The percentage adherence at each time point for each subject was determined by the following formula:

(# of pills dispensed - # of pills returned) / (# of elapsed days between dispense date and return date)

Two dichotomous variables of adherence by pill count were also created for each time point, based on whether the percent adherence by pill count was above the threshold levels of 70% and 85%.

A summary measure of the mean percent adherence by pill count for each subject was calculated by taking the mean of percent adherence for all visits for which pill count data was available for that subject

In data analysis, some subjects were evaluated as more than 100% compliant by the above formula. This could occur if women returned the pill bottle containing fewer doses than were expected given the time interval. Since it is not possible to be more than 100% adherent, we assigned each of these subjects a 100% adherence for the corresponding time interval and recorded elsewhere in the dataset that these values were originally over 100%.

Adherence by serum metabolite levels

Adherence by serum metabolite levels was recognized as difficult to define. While there is a model in existence which calculates the predicted change in serum 25-OHD for different doses of vitamin D supplement (31), this model was developed using data from men, and its applicability to a population of pregnant females is debatable. Therefore, we were faced with the challenge of establishing novel criteria for vitamin D supplement adherence by serum 25-OHD levels among pregnant females.

The 25-OHD level for each patient had been obtained at each monthly visit. In general, women have been found to have lower 25-OHD serum levels than men (20). This has been further elucidated by research showing that after adjusting for differences in season at enrollment, percent body fat, exercise habits, and age, the vitamin D levels of men are approximately 24% greater than those of women in a cross-sectional study

(32). While there is no data comparing how women and men respond to identical doses of vitamin D_3, these studies were thought to provide reasonable expectations of serum metabolite-based adherence thresholds.

According to the Heaney regression model (31), each additional 40 IU of vitamin D_3 ingested on a daily basis will elevate the circulating 25-OHD levels by approximately 0.28 ng/mL in adult males. After calculating the predicted serum 25-OHD for the doses used in this study and decreasing these values by 24% to adjust for gender differences, it was determined that values of 10.6 ng/mL and 21.2 ng/mL increases in serum 25-OHD from baseline would be used as thresholds to define subject adherence to doses of 2000 IU and 4000 IU, respectively.

Variable Definitions:

- Assigned dose is categorized by the treatment group to which each subject belongs. Both subjects and clinicians are blinded to the assigned dose, which is one of three options: 400IU, 2000IU, or 4000IU.

- Maternal race, marital status, and highest education level were determined by self-identification on a questionnaire administered at enrollment.

- Maternal age was calculated as self-reported date of birth subtracted from date of enrollment.

- Season at enrollment was determined by season (Winter, Spring, Summer, Fall) at first visit date.

- Initial BMI was calculated by trained staff based on each subject's measured height and weight at her first visit date.

- Skin pigmentation was determined by the first available measure of skin pigmentation in the inner arm as measured by the Smart Probe 400. The median value was determined, and a dichotomous variable was created defining women as either darker or lighter than the median subject skin pigmentation.

Confounding Variables: We investigated for the presence of confounding by performing bivariate analyses. In these analyses (**Table 1**), dosing groups were shown to be well-randomized with respect to race, age, season at enrollment, skin pigmentation, marital status, education level, and initial BMI.

Table 1: Demographic comparison of dosing groups to assess randomization

Covariate	Level	400IU n= 92	2000IU n=92	4000IU n=87	Chi-square test p-value
Maternal race	Black	14 (15%)	21 (22%)	19 (22%)	0.57
	Latina	42 (46%)	43 (47%)	36 (41%)	
	White	36 (38%)	28 (29%)	32 (33%)	
Maternal age	<20	9 (10%)	0 (0%)	7 (8%)	0.12
	20- <25	24 (26%)	29 (32%)	25 (29%)	
	25- <30	30(33%)	27 (29%)	27 (31%)	
	30+	29 (32%)	36 (39%)	28 (32%)	
Season	Spring	28 (30%)	29 (32%)	29 (33%)	0.94
	Summer	21 (23%)	24 (26%)	20 (23%)	

Table 1--continued

	Fall	20 (22%)	22 (24%)	17 (20%)	
	Winter	23 (25%)	17 (18%)	21 (24%)	
Skin pigmentation	Lighter than median	37 (57%)	32 (43%)	37 (55%)	0.21
	Darker than median	28 (43%)	42 (57%)	30 (45%)	
Marital status	Married	53 (58%)	49 (53%)	49 (57%)	0.81
	other	39 (42%)	43 (47%)	37 (43%)	
Maternal Education	< high school	18 (20%)	19 (21%)	13 (15%)	0.51
	HS grad	14 (16%)	14 (15%)	14 (17%)	
	Some college/AA	24 (27%)	34 (37%)	31 (37%)	
	Bachelors	12 (14%)	5 (5%)	12 (14%)	
	Postgraduate education	20 (23%)	19 (21%)	14 (17%)	
BMI	Median (IQR)	25.2 (8)	25.8 (8)	25.2 (9)	0.6*

*p-value of Kruskal-Wallis test (BMI followed non-normal distribution)

Statistical Analyses

First, a series of pairwise comparisons was performed in order to assess any demographic or baseline variable differences between treatment groups (**Table 1**). For all analyses in this study, 0.05 was the significance level threshold.

The data were also examined for bivariate associations of adherence by serum measures with demographic variables. Graphical representations of data were created

and examined for trends in adherence by the serum metabolite measures as categorized by age, race, dosing group, initial BMI, and season of enrollment (**Figures 1-5**).

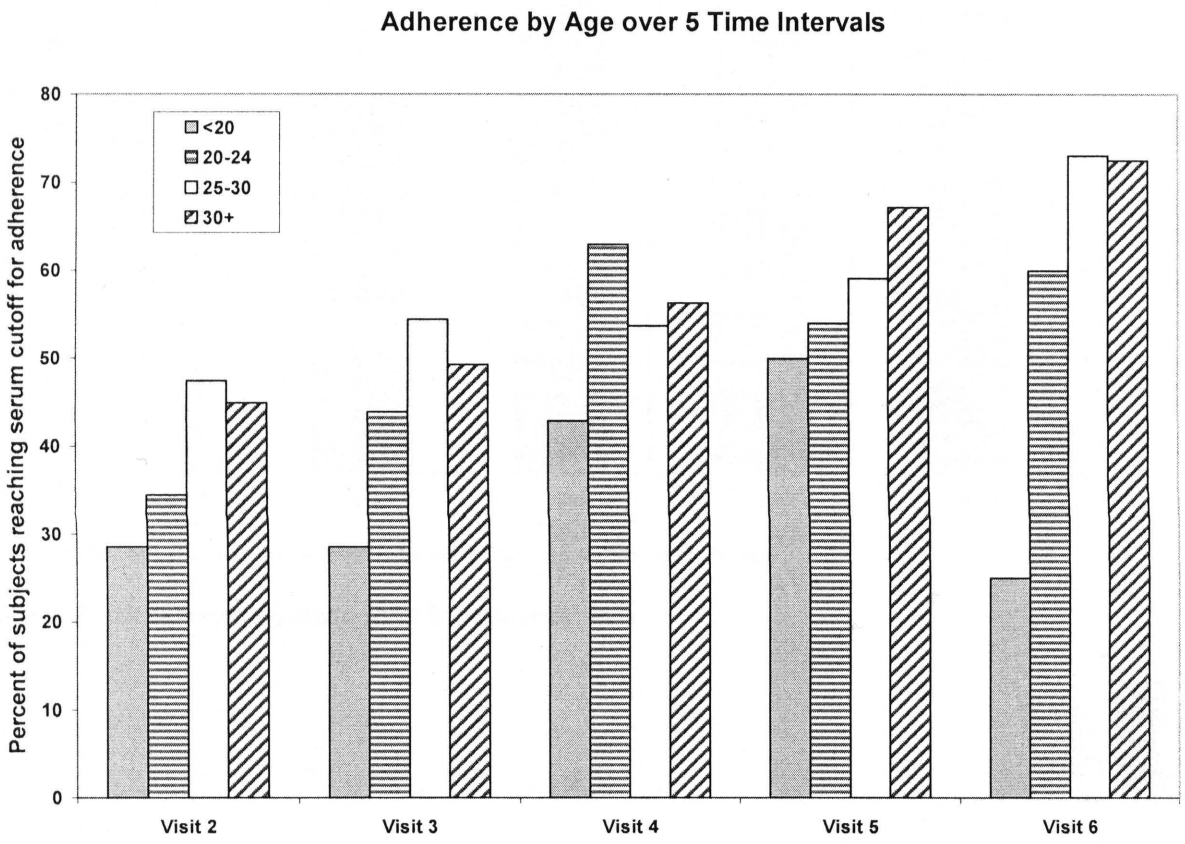
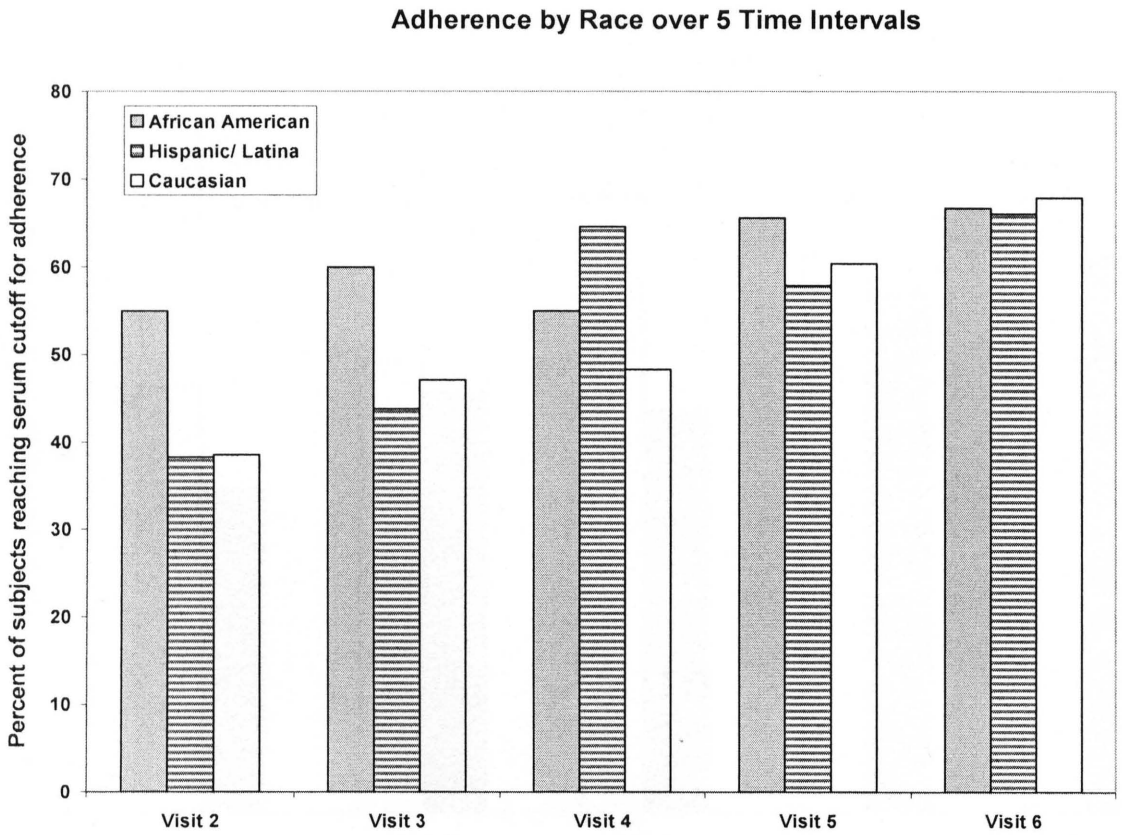
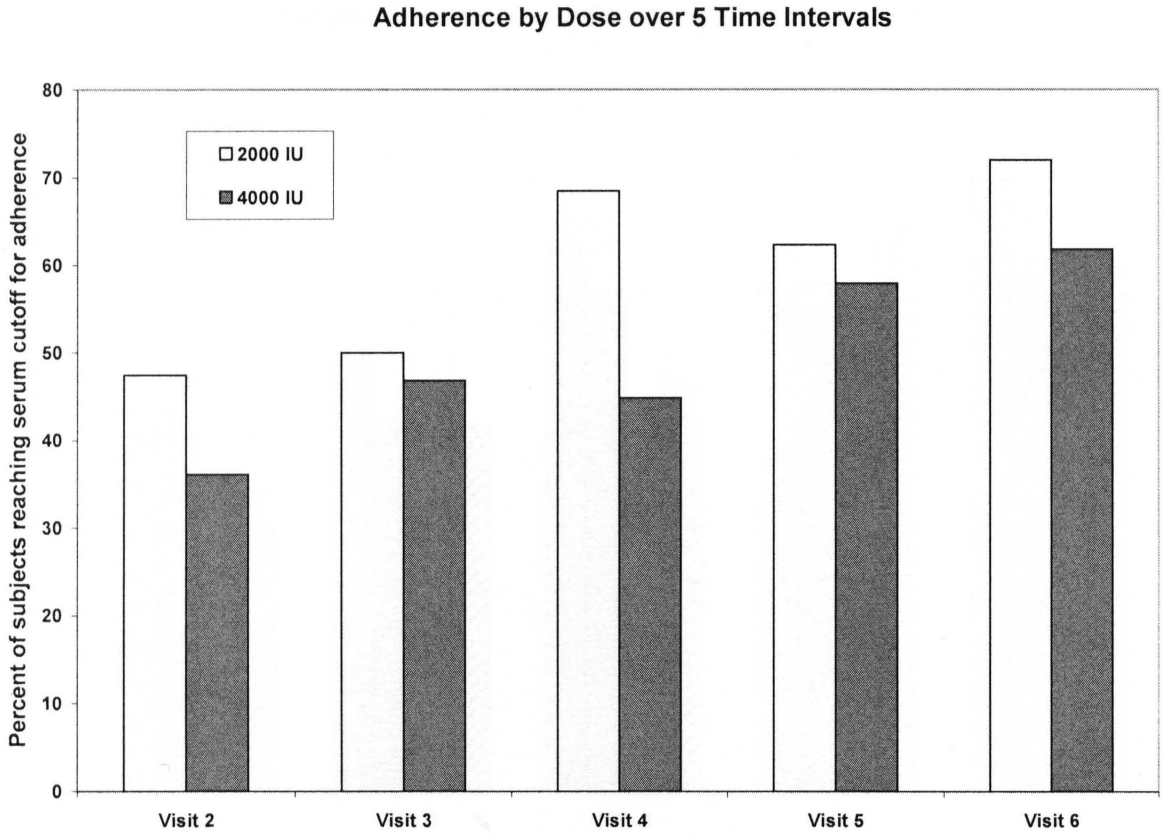


Figure 1--Adherence by Age over 5 Time Intervals



* Note: 100% was used as a maximum value for adherence; see section on limitations

Figure 2--Adherence by Race over 5 Time Intervals



Mantel-Haenzel Chi-Square p-value	0.11	0.66	0.001	0.57	0.27

Figure 3--Adherence by Dose over 5 Time Intervals

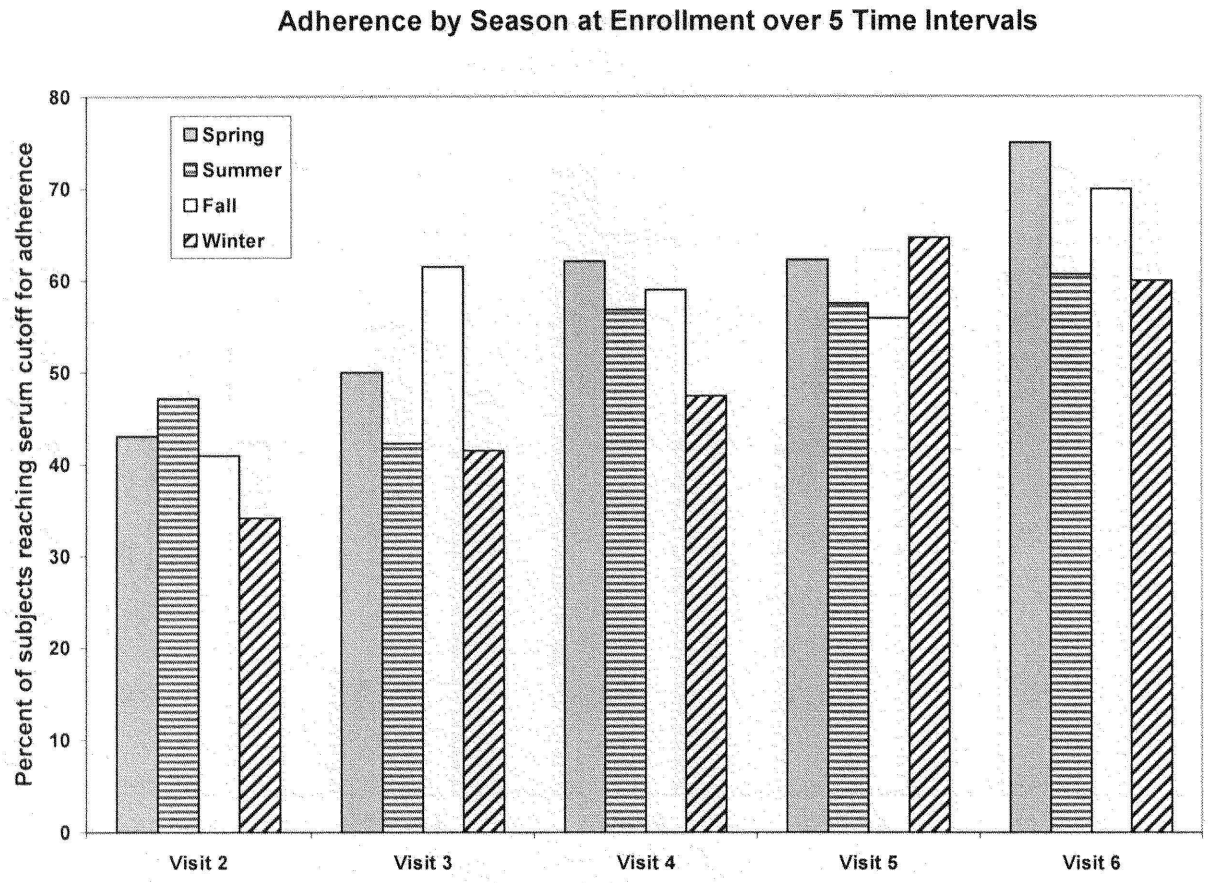


Figure 4--Adherence by Season at Enrollment over 5 Time Intervals

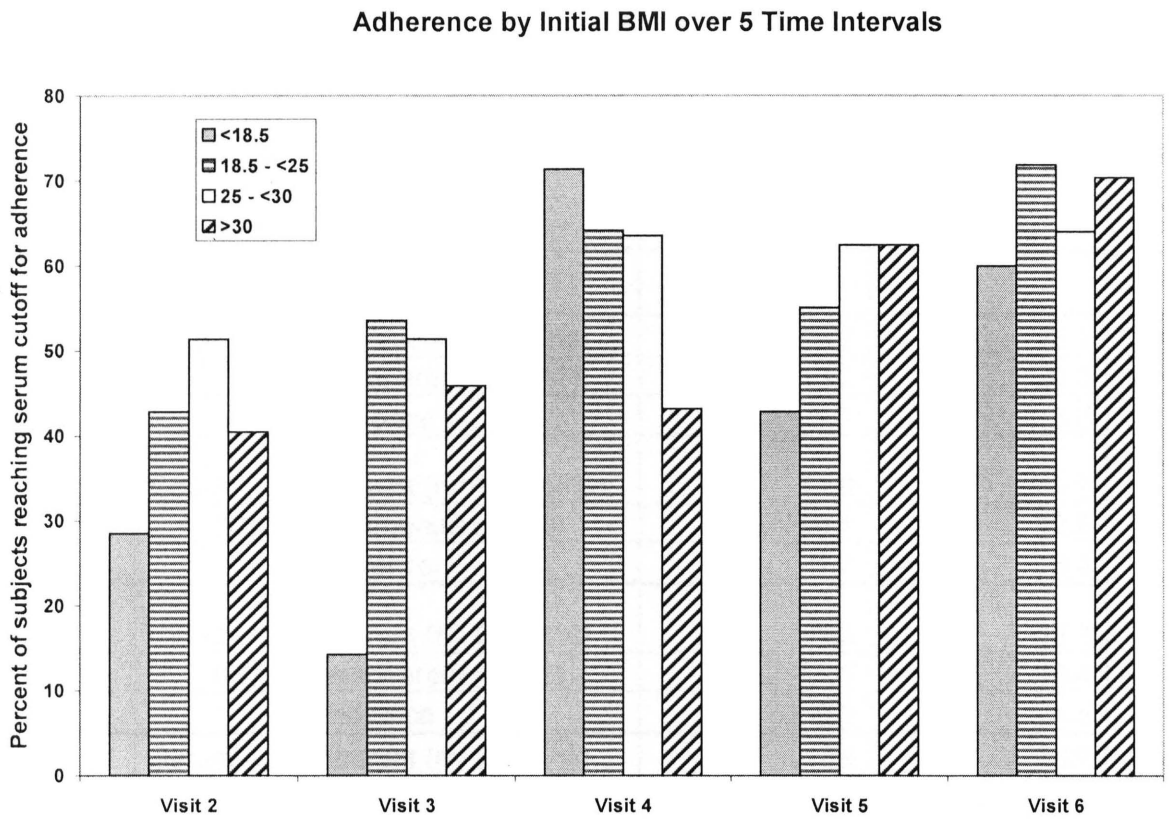


Figure 5--Adherence by Initial BMI over 5 Time Intervals

Data was compiled to examine trends in the change in 25-OHD from baseline to each visit in adherent and nonadherent groups as classified by the serum 25-OHD thresholds, as well as two standards of adherence as measured by pill count, 70% and 85% (**Table 2**). Graphical representations of these data were also created (**Figures 6-7**).

Table 2: Change in 25OHD from baseline of adherent/nonadherent subjects over 5 time intervals by 3 definitions of adherence

Visit number	Definition		mean change in baseline to	25OHD from steady state
			2000IU	4000IU
Visit 2	Serum	adherent (n= 80, 43%)	18.3	25.3
		nonadherent (n= 107)	13.2	20.3
		Difference	5.1	5.0
	Pill Count	adherent (n= 127, 68%)	11.8	19.2
	70%	nonadherent (n= 60)	10.1	12.8
		Difference	1.7	6.4
	Pill Count	adherent (n= 95, 51%)	11.3	18.2
	85%	nonadherent (n= 92)	11.1	16.4
		Difference	0.2	1.8
Visit 3	Serum	adherent (n= 92, 50%)	23	29
		nonadherent (n= 93)	12.8	20
		Difference	10.2	9.0
	Pill Count	adherent (n= 132, 71%)	12.7	21.9
	70%	nonadherent (n= 53)	13.4	18.9
		Difference	-0.7	3.0
	Pill Count	adherent (n= 109, 59%)	12.7	22.6
	85%	nonadherent (n= 76)	13.1	18.9
		Difference	-0.4	3.7
Visit 4	Serum	adherent (n=102, 58%)	31.6	34.1
		nonadherent (n=73)	13.7	21.5
		Difference	17.9	12.6
	Pill Count	adherent (n= 117, 67%)	17.0	22.5
	70%	nonadherent (n= 57)	14.1	19.8
		Difference	2.9	2.7
	Pill Count	adherent (n= 98, 56%)	17.5	22.4
	85%	nonadherent (n= 76)	14.0	20.7
		Difference	3.5	1.7
Visit 5	Serum	adherent (n= 97, 64%)	19	27.1
		nonadherent (n=54)	9.6	15.3
		Difference	9.4	11.8
	Pill Count	adherent (n= 95, 72%)	16.5	27.5
	70%	nonadherent (n= 37)	15.4	20.0

Table 2--continued

		Difference	1.1	7.5
	Pill Count	adherent (n= 79, 60%)	16.7	27.9
	85%	nonadherent (n= 53)	15.5	21.5
		Difference	1.2	6.4
Visit 6	Serum	adherent (n= 70, 48%)	22.9	31
		nonadherent (n= 35)	10.4	16.6
		Difference	12.5	14.4
	Pill Count	adherent (n= 0)	*	*
	70%	nonadherent (n= 1)	*	*
		Difference	*	*
	Pill Count	adherent (n= 0)	*	*
	85%	nonadherent (n= 1)	*	*
		Difference	*	*

* Not comparable due to inadequate sample size

Change in Serum 25-OHD from Baseline over 5 Time Intervals by 3 Definitions of Adherence - 2000 IU Dose

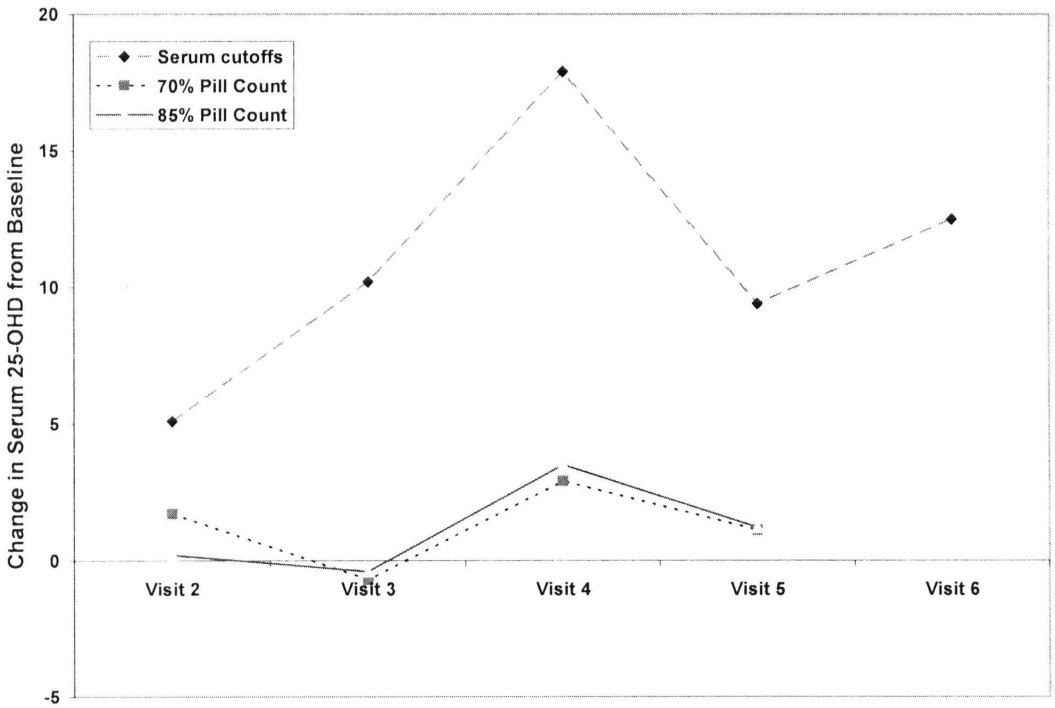


Figure 6--Change in Serum 25-OHD from Baseline over 5 Time Intervals by 3 Definitions of Adherence--2000 IU Dose

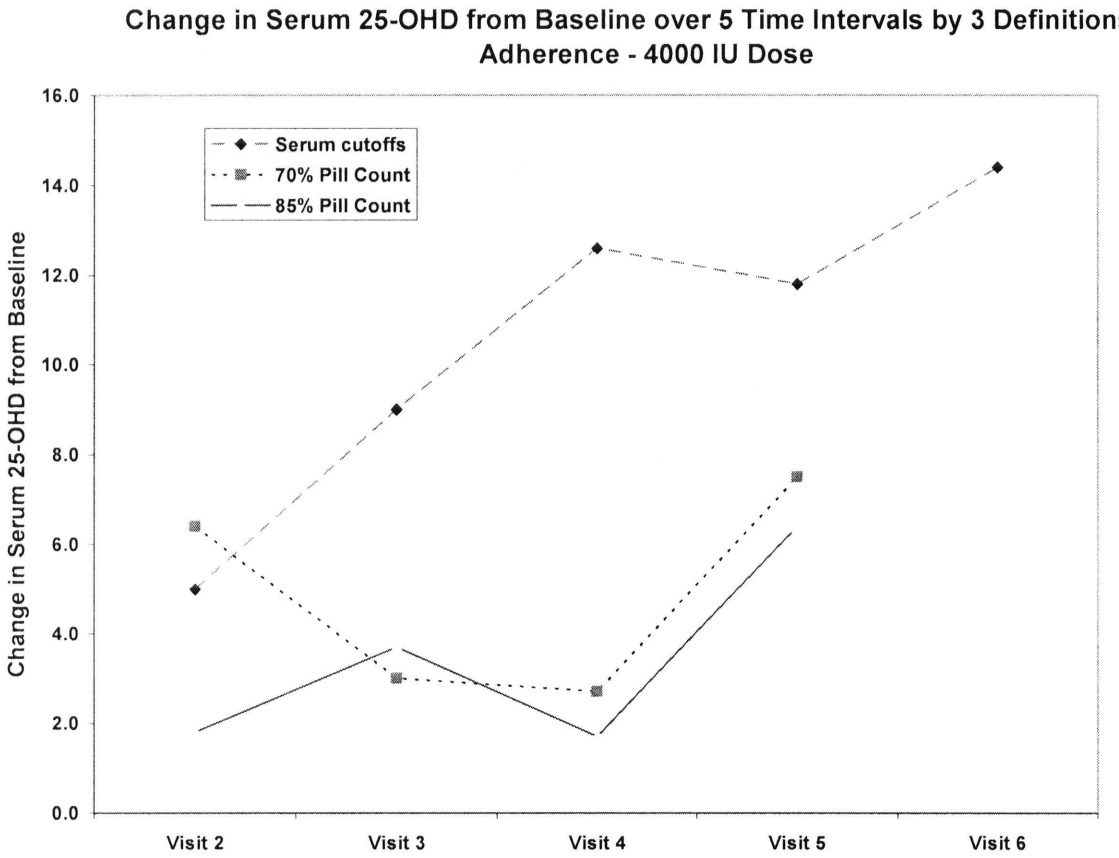


Figure 7--Changes in Serum 25-OHD from Baseline over 5 Time Intervals by 3 Definitions of Adherence--4000 IU Dose

A series of 5 multivariate logistic regression models were created to examine whether mean percent adherence by pill count was significantly associated with being adherent by serum 25(OH)D threshold while controlling for race, age, initial BMI and season at enrollment (**Table 3**).

Table 3: Odds ratios of the association of pill count with attainment of threshold serum values when *controlling for possible confounding variables*

Variable; OR; 95%CI; p-value	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Mean Pill Count Adherence	1.1	1.0	0.9	1.0	1.0
95% CI	0.9-1.3	0.8-1.3	0.6-1.2	0.7-1.3	0.7-1.4
P-value	0.57	0.75	0.48	0.85	0.84
Race					
overall p-value	0.17	0.31	0.11	0.29	0.95
Black v. White	2.9	2.5	2.5	3.3	1.4
95% CI	0.9-9.0	0.8-8.1	0.6-9.6	0.7-15.1	0.2-9.9
P-value	0.07	0.13	0.19	0.12	0.75
Hispanic v. White	1.4	1.5	3.7	2	1.2
95% CI	0.5-3.7	0.6-4.0	1.1-12.4	0.6-6.2	0.2-5.8
P-value	0.52	0.39	0.04	0.25	0.85
Dose (1 v. 2)	1.2	0.8	2.1	0.6	1.0
95% CI	0.5-2.6	0.4-1.8	0.9-5.2	0.2-1.6	0.3-3.0
P-value	0.69	0.58	0.10	0.34	0.97
Mom age in Yrs	1.0	1.1	1.0	1.1	1.1
95% CI	0.9-1.1	0.99-1.2	0.9-1.1	0.99-1.2	0.9-1.2
P-value	0.81	0.08	0.48	0.07	0.27
Season					
overall p-value	0.92	0.70	0.89	0.74	0.58
Fall v. Winter	1.1	1.4	0.7	0.7	1.2
95% CI	0.3-4.1	0.4-4.6	0.2-2.9	0.2-2.8	0.2-6.4
P-value	0.89	0.61	0.66	0.58	0.83
Spring v. Winter	1.10	0.70	1.20	1.00	1.6
95% CI	0.4-3.1	0.3-2.2	0.3-4.1	0.3-3.7	0.3-8.6
P-value	0.93	0.60	0.79	0.97	0.57
Summer v. Winter	1.4	0.8	1.0	0.6	0.6
95% CI	0.5-4.2	0.3-2.5	0.3-3.9	0.1-2.2	0.1-2.9
P-value	0.56	0.70	0.96	0.40	0.57
BMI	1.0	1.0	1.0	1.0	1.0
95% CI	0.9-1.03	0.96-1.02	0.9-1.03	0.97-1.1	0.96-1.1
P-value	0.47	0.59	0.22	0.41	0.48

Chapter 4 - Results

The three dosing groups included in this analysis were an active control group that was given supplements containing 400 IU of vitamin D_3 (twice the current Recommended Daily Allowance), a group taking 2000 IU, and a third group taking 4000 IU. Subjects in all groups were instructed to take one supplement once daily. A summary of pairwise comparisons of the demographics of dosing groups (**Table 1**), reveals that subjects in all dosing groups were not significantly different with respect to race, age, season at enrollment, initial inner arm skin pigmentation measurement, marital status, education level, or initial BMI.

The data were first examined for bivariate associations of compliance by serum 25(OH)D level with demographic variables. Graphical representations of data show percent of subjects reaching adherence by the serum metabolite adherence definitions as categorized by age, race, dosing group, initial BMI, and season of enrollment. While trends in age show a lower level of adherence in the youngest age group, no other consistent trends of adherence and age were evident (**Figure 1**). Race, season at enrollment and initial BMI showed no consistent trends (**Figures 2, 4-5**). While the data show a trend towards higher adherence by serum 25-OHD among the 2000 IU dosing

group as opposed to the 4000 IU dosing group, this only achieves the level of significance at visit 4 (**Figure 3**).

In terms of percentage adherence at each time point, the 70% adherence by pill count was the most generous standard, with percent adherence ranging from 68% to 72% of subjects at each time point. The 85% adherence by pill count standard and the established serum 25-OHD thresholds were comparable in the percentage of subjects classified as adherent, with ranges from 51-60% and 43-64%, respectively (**Table 2**). However, upon examining the mean difference in change in 25OHD level between adherent and nonadherent subjects for these standards, it is evident that the subjects classified as adherent under these two standards were very different. The mean change in 25(OH)D from baseline was greater for the group deemed adherent by serum standards than for the group classified as adherent by the 85% pill count standard at every time point. This was also represented graphically (**Figures 6-7**).

Finally, a series of 5 multivariate logistic regression models were created to examine whether mean percent adherence by pill count was significantly associated with the thresholds of adherence by serum 25(OH) D when controlling for race, dose, age, season at enrollment, and initial BMI. The analysis revealed that mean percentage of adherence by pill count was not a significant predictor of adherence by serum 25-OHD at any time point. Odds ratios ranged from 0.9 to 1.1, and a significance level of 0.05 was not reached at any time point (**Table 3**).

Chapter 5- Discussion

The importance of adherence to medication has been recognized in clinical trials and daily clinical practice alike. In order to properly evaluate the effects of medications, it is vital to determine whether they are taken as prescribed. The most frequent manner to evaluate adherence has been pill count, which offers advantages such as low cost and simplicity of collection and calculation, but yet has the disadvantages of frequently missing data and possible manipulation by subjects (11). In this trial, we sought to determine whether there was a significant association between adherence to vitamin D supplementation as measured by pill count and assessment of adherence based upon serum levels of vitamin D metabolites in a pregnant population.

The results of the present study could offer a new perspective on the determination of efficacy in clinical research. Currently, the efficacy of medications and supplements is determined primarily via randomized controlled clinical trials, in which subject adherence is assumed or measured via pill count. If there were an objective laboratory value by which adherence could more accurately be determined, studies could include the most adherent patients in the analysis to get a clearer picture of efficacy without the dilution of the treatment effect by nonadherent patients.

According to the multivariate logistic regression analysis performed there was not a significant association between adherence as defined by serum metabolite level and mean percent pill count adherence for any time point. This confirms previous studies which showed that pill count data are prone to errors (11). However, this analysis is unique in that it utilizes the pharmacokinetics of vitamin D metabolism to predict adherence, and the data are then compared to pill counts.

Another important finding of the analysis was a lack of a significant association between race and adherence. In a previous study of prenatal supplementation, non-Hispanic blacks were found to be less adherent than non-Hispanic whites (9). The lack of significance found in the present analysis could be due to the inclusion of Latina subjects, or could suggest that pill count data may overestimate the disparities of adherence among ethnic groups.

The study also offers an objective measure of adherence for vitamin D_3–containing supplements in women. Objective measures of known metabolites could be used more frequently in the clinic to monitor patient adherence to this and other prescribed regimens, much as HbA1C levels are currently used to monitor long-term blood glucose control in diabetic patients.

Strengths of this study include the collection of both pill count-based and serum metabolite-based adherence data at multiple time points. The study is strengthened by the consistency of findings across multiple time points. In addition, the subject population in this study was composed exclusively of pregnant women, which are

generally an adherent population, as was demonstrated by the success of a recent maternal folate supplementation program(29).

A final strength of this study was the foundation of the serum adherence thresholds of 25-OHD in the Heaney regression model (31). Of note, the predicted serum changes in 25(OH)D levels in this model were calculated on the basis of dose taken rather than dose prescribed. Therefore, they did make an effort to account for nonadherence in the study. However, the dose taken was based on patient pill count, so given the overestimation of adherence by pill count, there could still be some dilution of treatment effect in this model.

A limitation of the study was missing pill count data, which limited the sample size. In fact, 10% of the original subject population was eliminated from this analysis because the subjects did not have a single time point at which pill count data were available. The majority of patients did not have pill count data for all time points, meaning that their general level of adherence was essentially imputed from time points at which data were available. This may not be accurate, as an interim lack of adherence could have contributed to not returning the pill bottle at certain time points. There could also be unknown third factors, such as a more hectic schedule during certain months, which could both affect adherence and the likelihood of remembering to bring the pill bottle to the next appointment. The lack of data is a persistent problem in pill count adherence analyses, and one which can hopefully be alleviated in the future by changing the way adherence is measured in clinical trials.

Another limitation of this study was the lack of available data concerning the effects of a given oral supplementation dose of vitamin D_3 on the serum 25(OH)D levels in females as opposed to males. The study used to adjust the predicted serum adherence thresholds was a cross-sectional study of a population(32), not a controlled trial of supplementation doses. Therefore, the gender differences in 25(OH)D levels could have been due to confounding factors. However, we feel that the present study was based on the best information currently available.

A final limitation of the study was that using the formula for percent adherence by pill count, some subjects were found to be more than 100% adherent. This occurred when a subject returned a pill bottle containing less pills than anticipated given the time interval since the last visit. This raises a problem because taking more medication than prescribed is a form of nonadherence. It cannot be known whether more doses than prescribed were taken, or if these were spilled or consumed by other individuals. To deal with this problem, we assigned each of these subjects a 100% adherence for the corresponding time interval and recorded elsewhere in the dataset that these values were originally over 100%. Adherence by pill count therefore refers to returning the number or pills expected given the time interval or less. We believe that this was justified because there was also no upper limit on serum 25(OH)D levels, so subjects taking more supplement than prescribed would have been classified as adherent by this measure as well.

In conclusion, this study raises important questions about the utility of pill count in assessment of adherence in both clinical research and daily medical practice. Avenues for further research include the study of serum 25(OH)D levels in males and females taking an identical oral dose of vitamin D_3 and other, more innovative and reliable measures of subject adherence to prescribed regimens.

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